Biochemistry

AN ELECTROSTATIC FAULT AT THE ACTIVE SITE OF A PHOTORECEPTOR

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The blue light receptor photoactive yellow protein, a member of the PAS domain family, undergoes rhodopsin-like photochemistry through *trans* to *cis* photoisomerization of its *p*-coumaric acid (pCA) chromophore. An electrostatic fault exists in the PYP active site, where the normally basic chromophore remains ionized and is hydrogen bonded to the normally acidic protonated Glu46. The proton's unusual position is caused by the strongly shifted pK_a of these active site residues. Our goal is to quantify this electrostatic fault by measuring the pK_a of Glu46 in the ground state of PYP employing high pH jump rapid mixing absorbance spectroscopy. This approach allows us to kinetically separate between (1) the deprotonation of Glu46 to form a 400nm species and (2) the hydrolysis of the thiol ester bond linking the chromophore to Cys69. We are able to assign the formation of the 400nm species to the deprotonation of Glu46 due to the absence of such a transition in the high pH titration curve of E46Q PYP. Our current estimate of the Glu46 pK_a is 12. This result is important for understanding the mechanism by which PYP converts the energy of an absorbed photon into a signal-transducing protein structural change, since a central event in the PYP photocycle is a proton-hop from Glu46 to the chromophore. It shows that acidic side chains with unusually high pK_a values are not only important for energy transduction in the photosynthetic reaction center and bacteriorhodopsin, but also for a signal transduction in a water soluble receptor protein.